PCT/EP99/03403
University College London
Our Ref.: D 1583 PCT

Claims

1. An isolated or substantially pure form of a polypeptide having phosphatonin activity and an approximate molecular weight of 60 kDa as measured on SDS-PAGE.

2. The polypeptide of claim 1, which has an approximate molecular weight of 200 kDa as measured on bis- tris SDS-PAGE at pH 7.

3. The polypeptide of claim 1 or 2, which is glycosylated and/or phosphorylated.

4. The polypeptide of any one of claims 1 to 3, which is obtainable following purification from Saos-2 cells (Deposit No. ECACC 89050205).

5. The polypeptide of any one of claims 1 to 4 or an immunologically and/or biologically active fragment thereof, which comprises an amino acid sequence encodable by a polynucleotide selected from the group consisting of
   (a) polynucleotides encoding at least the mature form of the polypeptide comprising the amino acid sequence depicted in SEQ ID NO: 2 (Figure 8);
   (b) polynucleotides comprising the coding sequence as depicted in SEQ ID NO: 1 (Figure 8) encoding at least the mature form of the polypeptide;
   (c) polynucleotides encoding a polypeptide derived from the polypeptide encoded by a polynucleotide of (a) or (b) by way of substitution, deletion and/or addition of one or several amino acids of the amino acid sequence encoded by the polynucleotide of (a) or (b);
   (d) polynucleotides comprising the complementary strand which hybridizes with a polynucleotide of any one of (a) to (c);
   (e) polynucleotides encoding a polypeptide the sequence of which has an identity of 60% or more to the amino acid sequence of the polypeptide encoded by a polynucleotide of any one of (a) to (d);

(f) polynucleotides encoding a polypeptide capable of regulating phosphate metabolism comprising a fragment or an epitope-bearing portion of a polypeptide encoded by a polynucleotide of any one of (a) to (e);

(g) polynucleotides encoding an epitope-bearing portion of a phosphatonin polypeptide comprising amino acid residues from about 1 to 40, 141 to 180 and/or 401 to 429 in SEQ ID NO: 2 (Figure 8);

(h) polynucleotides comprising at least 15 nucleotides of a polynucleotide of any one of (a) to (g) and encoding a polypeptide capable of regulating phosphate metabolism;

(i) polynucleotides encoding a polypeptide capable of regulating phosphate metabolism comprising the cell and/or glycosaminoglycan attachment motif and/or the bone mineral motif of a polypeptide encoded by a polynucleotide of any one of (a) to (h); and

(j) polynucleotides the nucleotide sequence of which is degenerate as a result of the genetic code to a nucleotide sequence of a polynucleotide of any of (a) to (i).

6. The polypeptide of any one of claims 1 to 5, which is capable of regulating phosphate metabolism.

7. An isolated polynucleotide encoding a polypeptide of any one of claims 1 to 6.

8. The polynucleotide of claim 7, which comprises RNA or DNA.

9. The polynucleotide of claim 7 or 8, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

10. A polynucleotide which hybridizes with the polynucleotide of any one of claims 7 to 9 and which encodes a mutated version of the polypeptide of any one of claims 1 to 6 which has lost at least part of its phosphatonin activity.

11. A vector containing the polynucleotide of any one of claims 7 to 10.

12. A host cell genetically engineered with the polynucleotide of any one of claims 7 to 10, the vector of claim 11 or produced by introducing a expression control sequence into a host cell which mediates the expression of a gene encoding the polypeptide of any one of claims 1 to 6.

13. A process for isolating a phosphatonin polypeptide comprising the steps of:
    (a) culturing tumor-conditioned media or osteosarcoma cells to confluence in serum supplemented media (DMEM Eagles/10% FCS/glutamine/antimycotic (DMFCS);
    (b) incubating the cells on alternate days in serum free media DMEM Eagles/glutamine/antimycotic antibiotic (DM) up to five hours;
    (c) collecting conditioned serum free media from the cells and equilibrating the conditioned media to 0.06M sodium phosphate pH 7.2 and 0.5 M NaCl (PBS);
    (d) subjecting the media from (c) to an equilibrated column of concanavilin A sepharose;
    (e) washing the column extensively with PBS;
    (f) eluting the concanavalin A column with PBS supplemented with 0.5 M α-methyl-D-glucopyranoside;
    (g) subjecting the eluted material from (f) to cation exchange chromatography; and
    (h) eluting phosphatonin polypeptide containing fractions with 0.5 M NaCl.

14. A process for producing a polypeptide having the biological and/or immunological activity of phosphatonin comprising: culturing the host cell of claim 12 and recovering the polypeptide encoded by said polynucleotide from the culture.

15. A polypeptide which is obtainable by the process of claim 13 or 14 or by proteolytic cleavage of a phosphatonin polypeptide of any one of claims 1 to 6 or obtainable by the process of claim 13 or 14 by a PHEX metallopeptidase.

16. The polypeptide of any one of claims 1 to 6 or 15 having at least one of the following activities:

(a) it is capable of down-regulating sodium dependent phosphate co-transport;

(b) it is capable of up-regulating renal 25-hydroxy vitamin D3-24-hydroxylase; and/or

(c) it is capable of down-regulating renal 25-hydroxy-D-1-α-hydroxylase.

17. The polypeptide of any one of claims 1 to 6 or 15 having at least one of the following activities:

(a) it is capable of up-regulating sodium dependent phosphate co-transport;

(b) it is capable of down-regulating renal 25-hydroxy vitamin D3-24-hydroxylase; and/or

(c) it is capable of up-regulating renal 25-hydroxy-D-1-α-hydroxylase.

18. The polypeptide of claim 15 which has lost at least one of the activities as defined in claims 16 or 17.

19. An isolated antibody that binds specifically to the isolated polypeptide of any one of claims 1 to 6 or 15 to 18.

20. A nucleic acid molecule of at least 14 nucleotides in length hybridizing specifically with a polynucleotide of any one of claims 7 to 10 or with a complementary strand thereof.

21. An isolated regulatory sequence of a promoter regulating the expression of a nucleic acid molecule comprising a polynucleotide of any one of claims 7 to 10.

22. A recombinant DNA molecule comprising the regulatory sequence of claim 21.

23. A method for treating a medical condition related to a disorder of phosphate metabolism which comprises administering to a mammalian subject a therapeutically effective amount of the polypeptide of any one of claims 1 to 6 or 15 to 18 or of the polynucleotide of any one of claims 7 to 10, the vector of claim 10 or of the antibody of claim 19.

24. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject related to a disorder of phosphate metabolism comprising:
    (a) determining the presence or absence of a mutation in the polynucleotide of any one of claims 7 to 10; and
    (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

25. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject related to a disorder of phosphate metabolism comprising:
    (a) determining the presence or amount of expression of the polypeptide of any one of claims 1 to 6 or 15 to 18 in a biological sample; and
    (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

26. A method for identifying a binding partner to a phosphatonin polypeptide comprising:
    (a) contacting a polypeptide of any one of claims 1 to 6 or 15 to 18 with a compound to be screened; and
    (b) determining whether the compound effects an activity of the polypeptide.

27. A method of identifying and obtaining a drug candidate for therapy of disorders in phosphate metabolism comprising the steps of
    (a) contacting the polypeptide of any one claims 15 to 18 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to phosphate uptake, with said drug candidate to be screened under conditions to permit phosphate metabolism, and
    (b) detecting presence or absence of a signal or increase of the signal generated from phosphate metabolism, wherein the presence or increase of the signal is indicative for a putative drug.

28. A method of producing a therapeutic agent comprising the steps of the method of any one of claims 25 to 27; and
    (i) synthesizing the compound obtained or identified in step (b) or an analog or derivative thereof in an amount sufficient to provide said agent in a therapeutically effective amount to a patient; and/or
    (ii) combining the compound obtained or identified in step (b) or an analog or derivative thereof with a pharmaceutically acceptable carrier

29. An activator/agonist or inhibitor/antagonist of phosphate metabolism or binding partner of phosphatonin obtained by the method of any one of claims 25 to 27.

30. A composition comprising a polypeptide of any one of claims 1 to 6, or 15 to 18, the polynucleotide of any one of claims 7 to 10, a vector of claim 11, an antibody of claim 19, the nucleic acid molecule of claim 20 or the activator/agonist, inhibitor/antagonist or binding partner of claim 29.

31. The composition of claim 30 which is a pharmaceutical composition and further comprises a pharmaceutically acceptable excipient, diluent or carrier.

32. The composition of claim 31 which is a diagnostic composition and further comprises means for detection.

33. Use of a polypeptide of any one of claims 1 to 6 or 15 to 18 or a DNA encoding and capable expressing said polypeptide or the activator/agonist, binding partner of claim 29 or the antibody of claim 19, for the preparation of a medicament for treatment of a disorder of phosphate metabolism.

34. Use of a polypeptide of any one of claims 1 to 6 or 15, 16 or 18 or a DNA encoding and capable expressing said polypeptide, the activator/agonist or binding partner of claim 29 or the antibody of claim 19, for the preparation of a medicament for the treatment of hyperphosphatemia.

35. Use of a polypeptide of any one of claims 1 to 6, 15, 16 or 18 or a DNA encoding and capable expressing said polypeptide or the activator/agonist,

binding partner of claim 29 or the antibody of claim 19, for the preparation of a medicament for the treatment of renal osteodystrophy, hyperphophatemia in renal dialysis/pre-dialysis, secondary hyperparathyrodism or osteitis fibrosa cystica.

36. Use of a polypeptide of any one of claims 1 to 6, 15, 17 or 18 or a DNA encoding and capable expressing said polypeptide, the antibody of claim 19, the nucleic acid molecule of claim 20 or the inhibitor/antagonist of claim 29, for the preparation of a medicament for the treatment of hypophosphatemia.

37. Use of a polypeptide of any one of claims 1 to 6, 15, 17 or 18, or a DNA encoding and capable expressing said polypeptide, the antibody of claim 19, the nucleic acid molecule of claim 20 or the inhibitor/antagonist of claim 29, for the preparation of a medicament for the treatment of X-linked hypophosphatemic rickets, hereditary hypophosphatemic rickets with hypercalcuria (HHRH), hypomineralised bone lesions, stunted growth in juveniles, oncogenic hypophosphatemic osteomalacia, renal phosphate leakage, renal osteodystrophy, osteoporosis, vitamin D resistant rickets, end organ resistance, renal Fanconi syndrome, autosomal rickets, Paget's disease, kidney failure, renal tubular acidosis, cystic fibrosis or sprue.

38. Use of a polypeptide of any one of claims 1 to 6, 15, 17 or 18, or a DNA encoding and capable expressing said polypeptide, the antibody of claim 19, the nucleic acid molecule of claim 20 or the inhibitor/antagonist of claim 29, for the manufacture of a medicament for the treatment of a bone mineral loss disorder.

39. Use of a polypeptide of any one of claims 1 to 6, 15, 17 or 18 and PHEX metallopeptidase for the manufacture of a combined preparation for simultaneous, separate or sequential use for the treatment of a disorder of phosphate metabolism.

40. Use of a transformed osteoblast or bone cell line capable of phosphatonin overexpression for the production of phosphatonin.